,omm.
Dr. Loosli
Dr. Sommers
Dr. Wyatt

CHRONIC PULMONARY DISEASES

#907
#871 - denied
#546A-7/69-7/72
#546 -7/66-7/68

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N.Y. 10022

Application For Research Grant

FEB 20 1973

Date: February 15, 1973

1. Name of Investigator(s): (include Title and Degrees)
Ines Mandl, Ph.D., Associate Professor of Reproductive Biochemistry

2. Institution & Address. Columbia University College of Physicians & Surgeons
630 West 168th Street
New York, N.Y. 10032

3. Short Title of Project:
Effect of Smoke Constituents on the Composition of Connective Tissue Proteins Elaborated In Vitro.

4. Proposed Starting Date:
May 1, 1973

5. Anticipated Duration of this Specific Study:
2 -3 years

6. Brief Descripton of Objectives or Specific Aims:
Tissue culture techniques will be used to observe the effect of smoke constituents on isolated cellular elements of the lung and the formation of connective tissue components. Connective tissue growth and molecular integrity is fundamental to the maintenance of the structure of the lung and its normal function. Relatively minor changes in the molecular architecture of the connective tissue proteins may impair resiliency and increase susceptibility to damage by proteolytic enzymes of leucocyte or macrophage origin. It has been observed in several laboratories including our own that the primary damage in obstructive lung disease involves disruption of the elastin framework which appears to be facilitated by familial predisposition of certain individuals coupled with environmental factors such as air pollutants and smoking. Tissue culture offers a unique opportunity to assess the relative contribution of different factors on deviations in composition or structure of biosynthesized intermediary and end products. The planned study will concentrate on qualitative differences in elastin and collagen on a molecular level. Established lines of diploid fetal fibroblasts and strains of lung epithelial and endothelial cells can be exposed to air pollutants and smoke constituents with only minor effect on growth and respiratory functions. These cells elaborate collagen and elastin under normal culturing conditions and inhibition of key enzymes has been shown to alter biosynthesis, secretion and final composition. The effects of smoke constituents on these parameters have not previously been studied, in part because methods of analysis were not sufficiently sensitive. Preliminary experiments indicate that such a study is now feasible. Recognition of mediators of biosynthesis of forms of elastin with diminished resistance to subsequent damage may allow modifications which would eliminate or neutralize the noxious effects.

7. Give a Brief Statement of your Working Hypothesis: Deviations in the molecular architecture of the susceptible tissue elements may predispose to disruption of the collagen-elastin framework of the lung in emphysema and related diseases. Such molecular defects may be due to genetic or environmental factors. In vitro culture techniques offer unique opportunities to assess the effects of smoke constituents on compositional and structural integrity of the connective tissue proteins produced by cellular biosynthesis.

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8. *Details of Experimental* Design and Procedures: (Attach Separate Pages)

Attached, pages 5 to 9

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

The principal investigator's laboratories on the 4th floor of the Francis Delafield Hospital Division of Columbia University College of Physicians & Surgeons are adequate and fully equipped to carry out all the procedures and analyses described in this proposal.

10. Additional Requirements:

A second amino acid analyser is at our disposal in the Department of Biochemistry and a ckman sequenator in the Department of Medicine. For the culturing of cell strains and isolated lines, Dr. Mary Parshley's complete facilities for growth and maintenance as well as photomicroscopy and time lapse photography will supplement our own.

Biographical sketches of all principal and professional personnel (append)

Attached, page 10

12. List of publications: (Five most recent as pertinent) (append)

Attached, page 11

R: REDACTED MATERIAL

13. Budget: (1st year)

A. Salaries (Personnel by names)	% time	Amount
Professional		
Ines Mandl, Ph.D.	25	
Stephen Keller, Ph.D.	20	
Carlton E. Blackwood, Ph.D.	10	
Mary S. Parshley, Ph.D.	10	
Obligatory Fringe Benefits (22% I.M., M.P.; 13% S.K., C.B.)		REDACTED
Technical		
Yvonne Hosannah, M.S. Biology	75	
Mabel Wong, B.S. Chemistry	50	
Elizabeth Culbert, Secretary	15	
Obligatory Fringe Benefits (13%)		
	Sub-Total	
B. Consumable Supplies (list by categories)		
Media, Tissue Culture Supplies		1,000
Glassware, Columns, Parts		1,000
Chemicals, Sephadex, Solvents		1,000
	Sub-Total	3,000
C. Other Expenses (itemize)		
Equipment Repair & Maintenance		500
Travel to Scientific Meetings		400
Journal Subscriptions, Xeroxing		200
	Sub-Total	1,100
D. Permanent Equipment (itemize)		
None		0
E. Overhead (15% of A+B+C)		5,050
	Total	$38,717

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	3,000	1,100	0	5,405	41,437
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature ____________
Director of Project Ines Mandl, Ph.D. R Telephone

Signature ____________
Business Officer of the Institution F.P. Putney, Ph.D. Telephone

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Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	Pulmonary Biochemical Characteristics in Respiratory Distress Syndrome of the Newborn.	NIH SCOR (Scope E of HL 14218 Neonatal Lung Center L.S. James, M.D., principal investigator).	31,222 p.a. 1972/3	6/1/71 - 5/31/76
Pending	Chemical Composition of Lung Connective Tissue Proteins	NIH Program Project Grant (Chemical Predisposition to Lung Injury. G.M. Turino, principal investigator).		

(8) Details of Experimental Design and Procedures.

To accomplish our aim of assessing the effect of various additives on the composition and structure of elastin elaborated by fibroblasts as well as endothelial and apithelial cells of the lung, the experimental design involves (a) culturing of the cells under optimal conditions and in the presence of individual smoke components; (b) isolation of elastin from the cells and from the culture medium and (c) determination of overall composition, sequences and primary structure of the elastin molecules synthesized by the cells. It is expected that results obtained will reflect the changes produced in vivo in individuals exposed to these agents over periods of years or in experimental animals treated with higher doses over shorter intervals. Evidence is accumulating that qualitative abnormalities in lung elastin predispose to disruption of the connective tissue framework and subsequent functional impairment. Such a relationship was suggested as early as 1961 by Pierce et al. (Ann. Int. Med. 55:210, 1961) for patients with primary pulmonary emphysema. More recently, Johanson et al. (J. Clin. Investig. 51:288, 1972) and Hoffman et al. (J. Appl. Physiol. 33:42, 1972) confirmed the association of elastin destruction in the lung with emphysema. At the same time, comparative analyses performed in our laboratory showed statistically significant differences in the amino acid composition, the prevalence of peptide sequences and the susceptibility to enzyme digestion between elastin isolated at autopsy from non affected lungs and from lungs of emphysematous subjects or animals in which experimental emphysema had been induced by proteolytic enzyme injection. (see Keller & Mandl, Proteolysis and Pulmonary Emphysema, C. Mittman, editor, Academic Press, 1972, p.251 and Mandl, et al. id. p.439).

The study of human tissues in adequate numbers, though desirable, meets with difficulties of obtaining specimens and documenting histories as well as separation of effects due to age, genetic factors and environmental constituents. Mittman et al. (Chest, 60:214, 1971) reported that amongst persons with intermediate serum levels of proteolytic enzyme inhibitors (heterozygotes), smokers were more likely than non-smokers to develop emphysema, but amongst homozygously deficient individuals, no difference could be discerned. Tissue culture offers an opportunity to overcome ambiguities and difficulties. It complements animal studies and has the added advantage of greater flexibility and economy and since human cells are involved, possibly a more representative model of the human situation.

Several groups before us have used tissue culture models to reveal effects of air pollutants and smoke components on fibroblasts and respiratory epithelium. Decreased rate of growth, morphological changes and impairment of respiratory function were monitored, but to the best of our knowledge no attempts have been made to find qualitative differences in the connective tissue proteins synthesized by the cells. It is well known through numerous studies of collagen biosynthesis by fibroblast strains that the absence or blockage of essential cofactors such as ferrous iron or ascorbic acid or certain enzyme deficiencies may prevent completion of intermediary steps so that the normal sequence is disrupted and underhydroxylated or non-glycosylated molecules accumulate intracellularly. Enzymes such as proline hydroxylase, lysine hydroxylase, glucosyl or galactosyl transferase and lysyl oxidase may well be inhibited by smoke constituents. Crosslinking is known to be prevented by lathyrogens and by penicillamine affecting respectively formation of aldehyde intermediates from lysine or hydroxylysine and condensation of the aldehydes to aldols or Schiff bases in the crosslinks characteristic of both collagen and elastin. Although collagen biosynthesis has been studied in

greater detail than that of elastin, many parallel steps have been recognized, the mechanism of crosslink formation of desmosine, isodesmosine and lysinonorleucine has been elucidated and lung cells in culture were shown to synthesize elastin. Naum and Martin (personal communication) have demonstrated elastin in cultures of a cell line derived from peripheral lung tissue of a 3-month old male C57BL mouse and the same investigators are at present trying to define some of the culture variables that affect elastin accumulation. Exploratory tests in our own laboratory have shown that 200-500 x 10^6 cells of a newly established fibroblast culture yielded adequate amounts of elastin to allow isolation of highly purified elastin from both the medium and the cells. This means that enough cells to define compositional and structural differences can be produced at reasonable cost and with available facilities.

We have also explored the feasibility of growing cells in cultures into which toxic substances have been introduced. A considerable literature exists on the effects of air pollutants and smoke constituents on death rate, morphological and functional changes. Indications are that cells in culture may be exposed to substances of considerable cytotoxicity for prolonged periods if concentrations are sufficiently low. Extensive studies conducted by the Leuchtenbergers include monitoring of the effect of tobacco smoke on the growth characteristics of the respiratory epithelium (Cancer Res., 29:862, 1969; Exp. Cell Res., 62:161, 1970; Nature, 234:227, 1971; Nature, 241: 137, 1973). Similar techniques were also used by Crocker et al. (Arch. Environm. Health, 10:240, 1965; Cancer Res., 28:906, 1968 and 30:1312, 1970). These authors observed effects of carcinogenic hydrocarbons on the ultrastructure of respiratory rat epithelium cells. Even earlier, Cooper and associates described alterations of growth of L cells exposed to smoke gases (Proc. Soc. Exp. Biol. Med., 110:11, 1962) and Thayer and Kensler (Science, 146:642, 1964) reported inhibition of growth and protein synthesis of KB cells by the gaseous phase of cigarette smoke. Green and Carolin (New Engl. J. Med., 276: 421, 1967) ascribed direct cytotoxic effects of smoke on pulmonary cells to alteration of surface activity due to abnormal metabolic processes of the cells. Time and concentration related responses of rodent tracheal rings exposed to tobacco products in vitro were measured by Donnelly (Proc. Tobacco and Health Conf. 1970, p.127). At the same conference, Sabharval and Bhalla (Proc. Tobacco and Health Conf. 1970, pp.97 & 128) described investigations of tobacco smoke components on plant cells in tissue culture. They found tissue culture a sensitive and rapid bioassay for benzopyrene which at concentrations of 10^{-8} M induced marked growth within five weeks although higher concentrations proved quite toxic. Unpublished observations by Parshley et al. established that 50,100 and even 200 ppm of No_2 introduced into culture media of human and rat lung epithelium by dissolving known amounts of $NaNo_2$ in an adaptation of the procedure of Rounds and Bils (Arch. Environm. Health 10:251, 1965) had little or no effect on cell count, growth rate or glycolysis. Large vacuoles appearing in the area of the Golgi apparatus indicated a possible effect on lipid metabolism. In another series of experiments, the respiratory poison HCN added to culture media in the form of KCN showed more drastic effects; 20 - 60 ppm inhibited growth by 80-100% on the third day, however, by day 7 normal cells began to recover. As expected, KCN stimulated glucose utilization and lactic acid production although the growth rate was decreased. Based on these results by us and by others, no undue difficulties are anticipated in obtaining cells with sufficient capability to synthesize connective tissue proteins after exposure to specific toxic components of tobacco smoke. Such exposure may change the relative proportion of elastin, collagen and mucopolysaccharides in analogy to the recently demonstrated increases in the production of soluble collagen and glycoprotein by scleroderma fibroblasts

(Leroy, J. Exp. Med. 135:1351, 1972) or it may affect amino acid composition to reflect the differences observed in vivo between elastin derived from individuals with distinct histories of smoking and of lung disease. The specific experimental protocol proposed for the three phases of this project follows:

(a) In vitro production of fibroblasts and epithelial cells under normal conditions and in the presence of smoke components:

The potential for synthesizing collagen, elastin and glycoproteins may be inherent in several cell types in lung parenchyma. Alveolar epithelial cells, endothelial cells and alveolar macrophages have greater capacities than cells of the same type in other parts of the body. Human fetal lung fibroblasts have been reported by Elsdale and Foley (J. Cell Biol. 41:298, 1969) to have markedly greater potential for production and organization of layers of cells and collagen in tissue culture than gut fibroblasts. It is therefore planned to include in the proposed study cellular elements of the lung and single cell types and to establish primary cultures of explanted small cubes of whole lung as well as monolayer cultures of pure strains of cells originating from a single cell of one type. The organ culture technique has been used by others, e.g. Crocker, Donnelly and the Leuchtenbergers (l.c.) to observe effects of air pollutants including tobacco smoke on growth characteristics of respiratory epithelium. Established strains of epithelial cells from fetal and adult human and other mammalian lung tissues are maintained in our laboratories in connection with other programs. They include the widely used diploid human fibroblast line Wi-38, established by Hayflick (Exp. Cell Res. 25:585, 1961), mouse fibroblasts 3T3 and 3T6, originally obtained from Dr. Howard Green and applied extensively to studies of collagen biosynthesis and the PR 105 strain developed by the Priests which gives high yields of connective tissue proteins. All of these elaborate elastin and will be compared. In addition, we have recently isolated a fibroblast strain which has not been well characterized as yet but produces relatively large amounts of elastin both intracellularly and secreted into the medium. Intracellular elastin can be visualized with specific stains and elastin in the medium monitored by paper chromatographic confirmation of the unique desmosine + isodesmosine spot at the origin. Elastin has been isolated from both cells and media as described under (b).

Lines of epithelium, endothelium and fibroblasts will be prepared by enzymatic digestion and mechanical isolation. Brief trypsin treatment releases predominantly fibroblasts which tend to overgrow other types of cells when planted in culture. Sections of lung will be washed with physiological solution, minced into small pieces, suspended in 0.25%trypsin and stirred 1 hr. Fibroblasts will then be freed and after centrifugation at low speed and removal of the supernatant, the cells will be resuspended at a concentration of 10^6 cells/ml in 10% fetal calf serum in Ham's medium F12 or 20% human placental serum in Parker medium 199 and incubated at 37°C in Carrel flasks or milk dilution bottles. To obtain epithelial cells in almost pure culture, the connective tissue elements are digested by collagenase. Following the trypsin treatment, the cells are resuspended in a 0.1% collagenase solution, spun for two more hours, filtered and planted. Isolated colonies which develop on the floor of flask cultures from one or more of the same type can be scraped off and subcultured. From these pure cultures lines originating from one cell may be obtained by digestion, suspension and dilution to not more than 100 cells per ml. The few colonies or clones which develop around these cells can then be isolated mechanically and subcultured. To study the effect of smoke components, different concentrations of selected constituents will be introduced into the medium. Benzopyrene, 10^{-6} M to 10^{-9} M will be investigated; nitrite will be dissolved in the culture medium in the form of $NaNO_2$ which was shown to produce a death rate of cells comparable to that of direct exposure to NO_2 gas. In the

latter case, the cell suspension will be inoculated first and after the cells have become attached to the vessel floor, the medium will be changed to introduce $NaNO_2$ in dosage ranges from 0-200 parts per million. Other individual components will be selected later in the program and introduced in a similar manner.

Tobacco smoke is said to contain at least 10,000 different substances. The components believed to be most responsible for the alleged toxic effects are polycyclic aromatic hydrocarbons. To confirm this impression and eliminate other constituents, fractionation of smoke condensations may be useful. We intend, therefore, to follow the fractionation procedure recently described by Bresch et al. (Proc. Soc. Exp. Biol. Med. 141:747, 1972) and assess the effect of some of the fractions on elastin biosynthesis. Smoke condensate will be dissolved first in methanol to remove polar substances and extracted twice with cyclohexane. The combined extracts will then be extracted twice with nitromethane to separate aliphatic compounds from unsaturated compounds, heterocyclic components and aromatic substances. The nitromethane phase will contain almost all the benzopyrene plus traces of different phenols and nicotine, although most of the nicotine will have been left in the methanol fraction. In order to render the components enriched in the nitromethane phase soluble in water and thus facilitate their incorporation in acqueous media, the authors bound it to ovalbumin. More than 90% of the residue bound to ovalbumin was removable by benzene resulting in enrichment of benzopyrene and other aromatic hydrocarbons which bound to ovalbumin. Further purification can be achieved by chromatography of the ovalbumin adduct dissolved in phosphate buffer on Sephadex G 150.

(b) Isolation of elastin from tissue culture cells and media:

Methods successfully applied to the isolation of elastin from human and animal lung parenchyma have been adopted to allow extraction of elastin from used up growth media and from the viable cells. Extraction with NaCl and lipid removal with butanol and acetone will be followed by boiling in hot alkali. This process has been shown to destroy collagen and associated proteins and should result in very pure elastin. Nevertheless, the procedure will be complemented by milder, less drastic techniques to guard against possible loss of incompletely crosslinked or altered elastin which may be partly soluble in NaOH. Some aliquots will be subjected to digestion with pancreatic elastase which will yield elastolytic breakdown products which can be analysed. Others may be subjected to collagenase which will leave the elastin intact and, at the same time, indicate the normalcy or otherwise of collagen produced. Newly formed elastin has also been reported to be closely associated with structural acidic glycoproteins. Urea-mercaptoethanol is said to dissociate the complexes and will therefore be added to a representative proportion of the tests. Presence of desmosine and isodesmosine and the high proportion of valine and virtually no hydroxyproline will prove that elastin is involved.

(c) Determination of compositional or structural differences in elastins synthesized under normal conditions and in the presence of potentially toxic smoke components:

It is our contention that the relative amounts of collagen and elastin are less revealing of fundamental changes in the connective tissue framework than chemical composition and sequences within the molecule, and we intend to focus on these parameters. For complete amino acid analyses on a Technicon amino acid analyzer, the connective tissue proteins will be subjected first to hydrolysis in 6N HCl at 110°C for 24 hrs. In addition to the presence of the unique amino acids,

desmosine and isodesmosine, the most characteristic feature of elastin is the large percentage of valine, 13.5 residues per 1000 in bovine ligamentum nuchae elastin, seven times as much as in collagen. Differences in valine and other non-polar aminoacid residues and resulting shifts in the relative percentage of neutral, basic and acidic amino acids in the isolated elastins will be recorded and relationships sought with developmental stages of elastin biosynthesis. In the collagen fractions, changes in the total amino acid composition, polar residues and hydroxyproline content will be studied.

To reveal differences in the amino acid sequences, aliquots of purified elastin from the same specimen will be subjected to enzymatic hydrolysis with pancreatic elastase and microbial elastases and to limited breakdown with alcoholic KOH and collagen will be digested with collagenase and cleaved with CNBr. The peptide products will be separated by fractionation on DEAE and SE-Sephadex and Bio-Gel P_2, Dowex 50 and various Sephadex columns. The elution profiles obtained will be compared and the combined fractions under each peak analyzed. N-terminal amino acids will be determined by Edman degradation, C-terminal residues by hydrazinolysis. Since preliminary experiments have indicated that regions enriched in desmosine are poor in valine, non-specific microbial proteases with high elastolytic activities which preferentially cleave valine and isoleucine containing sequences, e.g., thermolysin, will be used for further breakdown and sequential analysis of the neutral regions of elastin. For both collagen and elastin analyses, microsomal hog kidney aminopeptidase, which preferentially liberates dipeptides of the Gly-Pro type from Gly-Pro-X sequences (Hopsu-Havu and Glenner, Acta Chem. Scand. 22:299, 1968) and an E. Coli peptidase which releases N-terminal non-polar amino acids from sequences with proline in the penultimate position will also be applied. Special attention will be paid to differences in the location and distribution of unusual components, known crosslinking amino acids and valyl peptides and the ratios of polar to non-polar amino acid residues in each fraction.

(11) Biographical sketches of investigators:

Dr. Ines Mandl, R , received her early education in Cork, Eire and her M.S. (organic chemistry) and Ph.D. (protein chemistry) at the Polytechnic Institute of Brooklyn in R and R respectively. From 1945-1949 she worked as assistant and coworker of the late professor Carl Neuberg at New York University. In 1949 she joined Columbia University, Department of Surgery as research associate and chief chemist of an army-sponsored project on bacterial collagenases. Thereafter she was assigned to the Department of Microbiology and since 1959 the Department of Obstetrics and Gynecology. From 1956 to 1972 she has been Assistant Professor of Biochemistry and principal investigator on several government and non-government sponsored projects. Recently she has been promoted to Associate Professor of Reproductive Biochemistry. She is a member of numerous professional societies and to date author or coauthor of 77 original or review articles and 47abstracts of papers presented at scientific meetings. Her main research interests are the relationship of structure and function in connective tissue proteins and specific enzymes degrading the proteins. She is also Editor-in-Chief of Connective Tissue Research: An International Journal.

Dr. Stephen Keller, R is at present research associate in Biochemistry and in Obstetrics & Gynecology at Columbia University College of Physicians & Surgeons. He obtained his Ph.D. in Biochemistry at Rutgers University in R , his M.A. in Physiology from St. John's University in R and his B.S. in Chemistry at the City College of New York in R . From 1955 to 1959 he worked as a fellow in Biochemistry at the Boyce Thompson Institute in Yonkers, New York, under the direction of Dr. Richard Block. Since 1959 he has been in the principal investigator's laboratory at Columbia University. His main interests evolve around phycicochemical analyses of elastin moieties. He is experienced in protein and enzyme fractionation techniques and co-authored chapters in the Laboratory Manual of Analytical Methods of Protein Chemistry, edited by Alexander and Block as well as 33 research papers.

Dr. Carlton E. Blackwood, R , is at present research associate at the College of Physicians and Surgeons and also Associate Professor of Biology at Iona College, New Rochelle, New York. He obtained his B.S. in Chemistry at Long Island University in R attended Medical School at Montpellier University, France, from R , then returned to graduate work at New York University where he obtained his M.S. in Biology in R and his Ph.D. in Biology in 1962. Between 1954 and 1959 he worked at the Funk Foundation for Cancer Research and, since 1959, at Columbia University College of Physicians & Surgeons. His main interests revolve around studies of proteolytic enzyme systems in tissue cultures, animal models and human tissues. To date he has co-authored 17 published papers or abstracts.

Dr. Mary S. Parshley, REDACTED , is at present Assistant Professor of Anatomy assigned to the Department of Obstetrics & Gynecology. She obtained her A.B. from Smith College in R and M.A. and Ph.D. degrees in Anatomy from the University of Pennsylvania in R and R respectively. She has been associated with Columbia University College of Physicians & Surgeons since 1940 and is a recognized authority in the field of tissue culture. Her 43 publications to date include papers on tissue culture of adult tissues, studies of the behavior of normal and malignant cells in tissue culture under diverse conditions, and the establishment and maintenance of tissue culture cell lines.

(12) List of publications (five recent, most pertinent).

1. Turino, G., Senior, R., Garg. B. D., Keller, S., Levi, M. and Mandl, I.: Serum Elastase Inhibitor Deficiency and Alpha$_1$-antitrypsin Deficiency in Patients with Obstructive Emphysema. Science, 165:709, 1969.
2. Mandl, I., Keller, S., Hosannah, Y. and Blackwood, C. E.: Induction and Prevention of Experimental Emphysema. In: Pulmonary Emphysema and Proteolysis. Ed. by C. Mittman, Academic Press, 1972, p.439.
3. Keller, S. and Mandl, I.: Qualitative Differences Between Normal and Emphysematous Human Lung Elastin. In: Pulmonary Emphysema and Proteolysis. Ed. by C. Mittman, Academic Press, 1972. p.251.
4. Evans, H. E., Mandl, I. and Keller, S.: Respiratory Distress Syndrome: Serum Enzyme Inhibitor Levels and Lung Tissue Elastin Composition. In: Pulmonary Emphysema and Proteolysis. Ed. by C. Mittman, Academic Press, 1972. p.91.
5. Mandl, I., Keller, S. and Levi, M.: The Relationship Between the Antigenicity and Chemical Composition of Components of Elastin Digests. In: The Chemistry and Molecular Biology of the Intercellular Matrix. Vol.I, p.657, E. A. Balazs, Ed., Academic Press, 1970.